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Supplementary webappendix

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Supplementary Appendix

The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase I/II trial

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Content

Methods

The VEBCON Consortium

In September 2014, the World Health Organisation (WHO) brought together an African and European consortium (VEBCON, VSV-EBola CONsortium) to harmonize parallel phase I trials of the recombinant vesicular-stomatitis-virus-vectored vaccine expressing the Zaire Ebolavirus glycoprotein (rVSV-ZEBOV) and to provide results crucial for phase II/III trials relevant to the current outbreak of Ebola virus disease (EVD). VEBCON centres include Lambaréné (Gabon), Kilifi (Kenya), Hamburg (Germany) and Geneva (Switzerland), with laboratory support in Marburg (Germany), London (UK) and Geneva. Its members are: Selidji Todagbe Agnandji (Centre de Recherches Medicales de Lambaréné, Gabon, Institut für Tropenmedizin, Universitätsklinikum Tübingen, Germany) and Sanjeev Krishna (St George's University of London, UK, Institut für Tropenmedizin, Universitätsklinikum Tübingen, Germany, Centre de Recherches Medicales de Lambaréné Lambarene, Gabon); Peter G. Kremsner and Jessica S. Brosnahan (Institut für Tropenmedizin, Universitätsklinikum Tübingen, Germany, Centre de Recherches Medicales de Lambaréné, Gabon); Philip Bejon and Patricia Njuguna (Kenya Medical Research Institute, Kilifi, Kenya); Marylyn M. Addo (University Medical Center Hamburg, Germany); Stephan Becker and Verena Krähling (Institute of Virology, Marburg, Germany); Claire-Anne Siegrist and Angela Huttner (Geneva University Hospitals); Marie Paule Kieny, Vasee Moorthy, Patricia Fast, Kayvon Modjarrad, Barbara Savarese, Olivier Lapujade (World Health Organization, Geneva, Switzerland).

Sample size determination

In the effort to test the rVSV-ZEBOV candidate vaccine across a range of doses, the Consortium's overall sample size target was roughly 250 subjects, as WHO estimates had concluded that roughly 74 – 124 subjects would be needed in each dose arm to demonstrate a ≥ 2-fold difference in ZEBOV-GP-specific antibody titres. Study-specific samples sizes were then based primarily on local recruitment

capacity. Given its relatively large capacity, Geneva investigators were requested to include 115 of the required volunteers, with roughly 100 receiving vaccine and 15 placebo.

Context: study launch and hold

The Geneva trial was launched in November 2014 and initially compared two recombinant vesicular stomatitis virus-vectored Zaire-ebola glycoprotein (rVSV-ZEBOV) vaccine doses (10^7 pfu and $5x10^7$ pfu) and placebo.[1] A planned interim safety analysis was performed on 28 November 2014, 14 and nine days after the first four and 15 volunteers, respectively, had been injected; these comprised the run-in group and received 10^7 pfu rVSV-ZEBOV open-label. For the analysis, the Data Safety Monitoring Board (DSMB) was charged with reviewing solicited and unsolicited adverse events (AE), safety blood work analyses, and the occurrence and duration or rVSV viremia and/or shedding (see protocol), all of which were deemed to reflect an acceptable safety profile. Shortly thereafter, however, the unexpected emergence of arthritis in some vaccinees led to a temporary study hold on 9 December 2014. The generally mild symptomatology and overall favourable course of vaccinees with arthritis allowed for a resumption of the study, albeit with a substantially reduced rVSV-ZEBOV dose; injections with placebo or $3x10^5$ pfu ("low-dose") thus began in early January 2015.

Box 1. Pre-determined study holding rules (from study protocol, section 6.1)

General tenet

The study in its entirety may be discontinued prematurely with reasonable justification by the PI, Sponsor, the vaccine manufacturer, Swissmedic or an Ethics Committee with oversight responsibilities at any time (see below), and/or individual subjects may terminate their participation prematurely, or have their participation be terminated by the Investigator.

Specific holding rules

Solicited local adverse events:

- If more than 25% of injections (minimum 2 individuals) are followed by Grade 3 solicited swelling or pain or Grade 4 redness beginning within 3 days after injection (day of injection and 2 subsequent days) and persisting at Grade 3 (swelling or pain)/4 (redness) for > 48 to maximum 72 hours depending upon symptom severity and kinetics

Solicited systemic adverse events:

- If more than 25% of injections (minimum 2 individuals) are followed by Grade 3 solicited systemic AE (or Grade ≥ 3 physical observations as defined above beginning within 3 days after study injection (day of injection and 2 subsequent days) and persisting at Grade ≥ 3 for > 48 to maximum 72 hours depending upon symptom severity and kinetics

Unsolicited adverse events:

- If more than 25% of volunteers (minimum of 2 individuals) develop a Grade ≥ 3 unsolicited AE (including laboratory AE and physical observations) that is considered probably or definitely related to injection and persists at Grade 3 for > 48 to maximum 72 hours depending upon symptom severity and kinetics

A suspected unexpected serious adverse drug reaction (SUSAR) occurs that is life-threatening or results in death

Randomisation and masking of the low-dose group

Non-deployable subjects in the low-dose group were randomized 9:1 to either rVSV-ZEBOV or placebo, while deployable subjects received $3x10^5$ pfu open-label. Not including a placebo arm for deployable volunteers at risk of subsequent exposure to ebolavirus was a specific request of the World Health Organisation (WHO) and other Geneva-based international institutions (Doctors without Borders). A statistician not involved in the study analysis generated the randomization list at www.randomization.com using investigator-blinded, randomly permuted blocks of varying sizes prior to study resumption. Only the pharmacist, an infectious disease physician not involved in the study, and a laboratory technician had copies of the lists.

Clinical personnel and study participants were unaware of the treatment allocation of all non-deployable low-dose subjects. Through sample coding, specialized laboratory personnel performing specific assessments were blinded to the treatment administered. Through recoding of subject

numbers (performed by secuTrial®, Berlin, Germany), data analysts remained blinded to individual treatment allocations. Given the uneven number of subjects in each treatment arm, the study statistician (CC) was not blinded to group treatment allocation during analyses. Study investigators and subjects with arthritis or dermatitis became aware of allocation to vaccine as opposed to placebo whenever rVSV was detected in joint or skin samples. The blind was intentionally lifted on February 26, 2015 (after completion of the day-84 [D84] study visit) for the 11 subjects of the first high-dose cohort with arthritis, and on April 7, 2015 for all remaining subjects of the high-dose cohort. The blind was also intentionally lifted on April 7, 2015 for the 15 participants of the low-dose cohort with arthritis or skin lesions and on May 18 (completion of the D84 visits) for all subjects.

Vaccine reconstitution

The $3x10^5$ pfu dose was prepared by initial dilution of 1ml (10^8 pfu) to 10 ml (10^7pfu/ml), followed by dilution of 3ml to 10ml ($3x10^6$/ml), then dilution of 1ml to 5ml ($3x10^5$/0·5ml). Doses of 10^7 pfu or $5x10^7$ pfu were prepared as previously described.[1] Placebo syringes contained 0·5 ml normal saline (NS) and were packaged identically.

Monocyte activation

Human whole blood was treated with BD Pharm Lyse solution (BD Biosciences) to remove erythrocytes. White blood cells (WBC) were washed with 1% FCS and 0·1% sodium azide containing PBS and counted using the countess Automated cell counter (Life technologies). $1\text{-}2x10^6$ white blood cells were then stained with the following anti-human mAbs: CD169-PE (clone: 7-239); HLA-DR-PE/Dazzle594 (clone: L234); CD14-Alexa Fluor 700 (clone: M5E2); CD20-APC-Cy67 (clone: 2H7); CD3-APC-Cy7 (clone: SK7); CD8-APC-Cy7 (clone: SK1); CD19-APC-Cy7 (Clone: SJ25C1); CD16-Pacific Blue (clone: 3G8) (all from BioLegend); mouse IgG1k-PE isotype control (BD Biosciences). Monocytes were defined within the singlet population of FSCint and SSCint as dump- (CD3, CD8, CD19 and CD20) and

HLA-DR$^+$.[2] Three sub-populations of monocytes were then defined based on their expression of CD14 and CD16 (CD14$^+$CD16$^-$ "classical monocytes"; CD14$^+$CD16$^+$ "intermediate monocytes"; CD14$^-$CD16$^+$ "non-classical monocytes").[3] Rainbow Calibration Particles (8 peaks) (BD Biosciences) were used to calibrate the Gallios cytometer (Beckman Coulter) parameters before each data acquisition. Data were analysed using FlowJo Software (Tree Star). The CD169 expression analysis was performed calculating the ratio of geometrical mean fluorescence intensity values of CD169 expression and isotype control.

Immunogenicity - ZEBOV-GP-ELISA

ZEBOV-GP-specific antibodies assessed with the commercial ELISA assay from Alpha Diagnostic International (ADI, San Antonio, Texas, USA) were quantified according to the manufacturer's instructions (quantification method C). Sera were assessed by endpoint dilutions, starting at 1:100. Optical density (OD) was read at 450 nm, and samples immediately above and below the OD of 1·0 (experimentally defined as within the linear range of the assays) were used to convert ODs into ELISA units/ml, using the standards provided. The seropositivity threshold was defined by the geometric mean concentration (GMC) + 2 standard dilutions (SD) of day 0 samples from non-deployable subjects.

Other immunogenicity assays have been described elsewhere.[1]

Data analysis

Baseline demographics, clinical characteristics and adverse events

Categorical variables are described by counts and percentages, continuous variables by mean and standard deviations. Comparisons were performed using chi-squared tests (or Fisher exact tests when expected counts were lower than five) or by Mann-Whitney tests (or Kruskal-Wallis tests) for continuous factors for independent groups. In the absence of relevant differences between vaccines doses of 10^7 and $5x10^7$ pfu, their results are presented together ("high-dose subjects") and compared with those of patients receiving the low-dose ($3x10^7$ pfu). Similarly, placebo recipients recruited in the

first and second groups were pooled. Randomised participants were compared with open-label participants receiving the same vaccine dose. In the absence of relevant differences, randomised and open-label participants receiving the same vaccine dose were pooled. Clinical characteristic were compared between low- and high-dose participants and between low-dose vaccines and placebo recipients (Mann-Whitney test). Additionally, the changes from day 0 were compared between low-dose vaccines and placebo recipients (Mann-Whitney test). Intensity and frequency of adverse events occurring during the first 14 days were compared between low- and high-dose participants and between low-dose vaccinees and placebo recipients with Fisher's exact. Time to onset was described by categories and compared between low- and high-dose participants with Fisher's exact. Associations between the most frequent adverse events (AE) and pre-defined factors (rVSV in copy number/mL, rVSV >20 copies/mL, age, lymphopaenia, monocytosis, gender) were assessed by comparing the factors between participants with and without arthritis using the Mann-Whitney test for continuous factors and Chi-square or Fisher's exact test for categorical factors. In a post-hoc analysis, we explored whether the magnitude of association between vaccine dose and risk of arthritis was modified by age vaccinee age. The corresponding interaction term was tested in a logistic regression model. The risk of arthritis predicted by this logistic regression model was graphically represented.

Description of antibody titres

Titres are described by geometric means (logarithm base 10) and 95% confidence intervals. They are graphically represented by reverse cumulative distributions obtained by plotting, for each possible value of the titre (abscissa), the proportion of subjects with a titre greater than this value. Antibody titres were compared between days 0 and 28 with Wilcoxon's test for paired data. Seropositivity rates at days 0 and 28 were reported and compared between days 0 and 28 with the McNemar test. Titres, seropositivity and seroresponse rates were compared between low- and high-dose groups by Mann-Whitney and Fisher's exact tests.

Association between doses and GMT/GMC, seropositivity and seroresponse rates

A trend of the geometric mean, seropositivity and seroresponse rates across vaccine doses was tested using Cuzick's and Cochran-Armitage tests. Additionally, comparisons between low- and high-dose groups were conducted with Mann-Whitney and Chi-squared tests (or Fisher's exact test when expected counts were below five).

Association between participants' characteristics and GMT/GMC

Associations between geometric means and pre-defined factors (age, gender, peak viraemia, lymphopaenia, monocytosis, lymphocyte counts at day 1, monocytes counts at day 3, and number of AE) were assessed with linear regression models. In these models, the dependent variable was the logarithm (base 10) of the titre given data skew. Therefore, the regression coefficients represent mean differences in $\log_{10}$-titre and are expressed as ratios of geometric means (RGM) to facilitate the presentation of results. For categorical independent variables, the RGM represents the ratio of geometric mean compared with the reference category. For continuous independent variables, the RGM represents by how much the geometric mean is multiplied when the independent variable increases by one unit. The assessment of associations was performed using linear regression models in the low-dose group (unadjusted associations) and in all vaccinees (association adjusted for vaccine dose).

Correlations among antibody titres

Strength of associations between variables was assessed by Spearman's correlation coefficient.

All statistical tests were two-sided with a risk α of 0.05. Analyses were conducted with R software, version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

Table S1. Baseline demographic and clinical characteristics of randomised *vs* open-label (deployable) participants of the low-dose group.

		All	Non-deployable	Deployable	P value*
Group size	**n**	**56**	**43**	**13**	
Age (years)	Mean (SD)	40·4 (11·6)	39·6 (11·6)	43·0 (11·8)	0·326
	Missing data, n	0	0	0	
Gender**	Female, n (%)	30 (53·6)	23 (53·5)	7 (53·8)	1
	Male, n (%)	26 (46·4)	20 (46·5)	6 (46·2)	
Ethnicity**	Caucasian, n (%)	56 (100)	43 (100)	13 (100)	NA
	Other, n (%)	0 (0)	0 (0)	0 (0)	
Haemoglobin, g/L	Mean (SD)	143 (13·4)	143·5 (14)	141·2 (11·6)	0·749
	Missing data, n	0	0	0	
Platelets, G/L	Mean (SD)	249·6 (60·9)	253·3 (62·6)	237·2 (55·6)	0·522
	Missing data, n	0	0	0	
Leukocytes, G/L	Mean (SD)	6·4 (2·0)	6·4 (2·2)	6·3 (1·4)	0·749
	Missing data, n	0	0	0	
Lymphocytes, G/L	Mean (SD)	2·0 (0·6)	2 (0·6)	1·9 (0·4)	1
	Missing data, n	0	0	0	
Neutrophils, G/L	Mean (SD)	3·7 (1·7)	3·7 (1·8)	3·7 (1·1)	0·467
	Missing data, n	0	0	0	
Monocytes, G/L	Mean (SD)	0·5 (0·8)	0·5 (0·2)	0·5 (0·1)	0·938
	Missing data, n	0	0	0	
Creatinine (mg/dL)	Mean (SD)	73·5 (11·7)	71·7 (10·7)	79·6 (13·4)	0·135
	Missing data, n	0	0	0	
AST (U/L)	Mean (SD)	15·1 (5·5)	14·6 (4·9)	16·6 (7·1)	0·450
	Missing data, n	1	1	0	
ALT (U/L)	Mean (SD)	18·2 (8·4)	18·6 (8·9)	17 (6·8)	0·620
	Missing data, n	0	0	0	

*P values are for comparisons between deployable participants and non-deployable participants receiving low-dose ($3x10^5$ pfu) vaccine; the Mann-Whitney test was used for continuous variables and Chi-square or Fisher's exact tests for categorical variables.
**Values presented as absolute numbers (percentages).
AST: aspartate aminotransferase; ALT: alanine aminotransferase; NA: not applicable

Table S2. Baseline demographic and clinical characteristics of placebo recipients from both dose groups.

		All	Placebo (high-dose group)‡	Placebo (low-dose group)	P value*
Group size	**n**	**13**	**8**	**5**	
Age (years)	Mean (SD)	40·8 (12·2)	38·5 (11·9)	44·6 (13·2)	0·435
Gender (%)**	Female	7 (53·8)	4 (50)	3 (60)	1
	Male	6 (46·2)	4 (50)	2 (40)	
Ethnicity (%)**	Other	0 (0)	0 (0)	0 (0)	
	Caucasian	13 (100)	8 (100)	5 (100)	
Deployability (%) **	Non- depl.	13 (100)	8 (100)	5 (100)	
	Deployable	0 (0)	0 (0)	0 (0)	
Haemoglobin, g/L	Mean (SD)	142·1 (10·7)	141·5 (11·3)	143 (10·8)	1
Platelets, G/L	Mean (SD)	234·4 (60.5)	225·8 (56·4)	248·2 (71·0)	0·622
Leukocytes, G/L	Mean (SD)	5·7 (1.4)	5·7 (1·6)	5·6 (1·2)	0·943
Lymphocytes, G/L	Mean (SD)	2 (0·7)	2·0 (0·7)	2·0 (0·6)	0·833
Neutrophils, G/L	Mean (SD)	3·0 (1·0)	3·1 (1·1)	2·9 (1·0)	0·833
Monocytes, G/L	Mean (SD)	0·5 (0·1)	0·5 (0·1)	0·5 (0·2)	1
Creatinine (mg/dL)	Mean (SD)	73·6 (12·9)	74·9 (14·6)	71·6 (10·7)	0·833
AST (U/L)	Mean (SD)	16·1 (5·6)	16·1 (5·8)	16 (6·0)	0·941
ALT (U/L)	Mean (SD)	19.8 (12.9)	21.3 (14.7)	17·4 (10·5)	0·713

‡As previously described in reference 1.
*P values are for comparisons between recipients of placebo in the low *vs* high-dose groups; the Mann-Whitney test was used for continuous variables and Chi-square or Fisher's exact tests for categorical variables.
**Values presented as absolute numbers (percentages).
AST: aspartate aminotransferase; ALT: alanine aminotransferase

Local and systemic solicited adverse events

Table S3. Local and systemic solicited adverse events with onset ≤14 days after injection (all).

Event		Low-dose		High-dose‡		P values*	
		$3x10^5$ pfu (n=51)	Placebo (n=5)	$\geq 10^7$ pfu (n=51)	Placebo (n=8)	$3x10^5$ pfu *vs* placebo	$3x10^5$ pfu *vs* $\geq 10^7$ pfu
Any adverse event	None (%)	6 (11)	1 (20)	1 (2)	1 (13)	0·579	0·141
	Mild (%)	18 (35)	3 (60)	14 (27)	5 (63)		
	Moderate (%)	20 (39)	1 (20)	25 (49)	2 (25)		
	Severe (%)	7 (14)	0 (0)	11 (22)	0 (0)		
Erythema**	None (%)	50 (98)	5 (100)	50 (98)	8 (100)	1	1
	Mild (%)	0 (0)	0 (0)	1 (2)	0 (0)		
	Moderate (%)	1 (2)	0 (0)	0 (0)	0 (0)		
Swelling / induration**	None (%)	49 (96)	5 (100)	49 (96)	8 (100)	1	1
	Mild (%)	2 (4)	0 (0)	2 (4)	0 (0)		
Pain**	None (%)	40 (78)	2 (40)	12 (24)	8·(100)	0·094	**<0·0001**
	Mild (%)	11 (22)	3 (60)	38 (75)	0 (0)		
	Moderate (%)	0 (0)	0 (0)	1 (2)	0 (0)		
Objective fever	None (%)	50 (98)	5 (100)	38 (75)	8 (100)	1	**0·001**
	Mild (%)	1 (2)	0 (0)	13 (25)	0 (0)		
	Moderate (%)	0 (0)	0 (0)	0 (0)	0 (0)		
Subjective fever	None (%)	39 (76)	5 (100)	19 (37)	6·(75)	0·707	**0·001**
	Mild (%)	8 (16)	0 (0)	19 (37)	2 (25)		
	Moderate (%)	3 (6)	0 (0)	10 (20)	0 (0)		
	Severe (%)	1 (2)	0 (0)	3 (6)	0 (0)		
Chills	None (%)	36 (71)	3 (60)	24 (47)	8 (100)	0·656	0·100
	Mild (%)	8 (16)	1 (20)	11 (22)	0 (0)		
	Moderate (%)	5 (10)	1 (20)	12 (24)	0 (0)		
	Severe (%)	2 (4)	0 (0)	4 (8)	0 (0)		
Myalgia	None (%)	31 (61)	5 (100)	17 (33)	5 (63)	0·654	**0·036**
	Mild (%)	13 (25)	0 (0)	21 (41)	1 (13)		
	Moderate (%)	4 (8)	0 (0)	10 (20)	2 (25)		
	Severe (%)	3 (6)	0 (0)	3 (6)	0 (0)		
Headache	None (%)	33 (65)	4 (80)	21 (42)	5 (63)8	0·398	0·061
	Mild (%)	12 (24)	0 (0)	15 (29)	3 (38)		
	Moderate (%)	5 (10)	1 (20)	13 (25)	0 (0)		
	Severe (%)	0 (0)	0 (0)	2 (4)	0 (0)		
Fatigue	None (%)	17 (33)	3 (60)	20 (39)	6 (75)	0·520	0·386
	Mild (%)	18 (35)	2 (40)	11 (22)	2 (25)		
	Moderate (%)	14 (27)	0 (0)	19 (37)	0 (0)		
	Severe (%)	2 (4)	0 (0)	1 (2)	0 (0)		
Arthralgia§	None (%)	44 (86)	4 (80)	42 (82)	8 (100)	0·552	0·908
	Mild (%)	5 (10)	1 (20)	6 (12)	0 (0)		
	Moderate (%)	2 (4)	0 (0)	2 (4)	0 (0)		
	Severe (%)	0 (0)	0 (0)	1 (2)	0 (0)		

‡As previously described in reference 1.

*P values are for comparisons of solicited local and systemic adverse events between placebo recipients and vaccinees in low-dose and high-dose groups: Fisher's exact test was used.

**At injection site.

§Arthralgia was solicited only in the low-dose group.

Table S4. Local and systemic solicited AE within 14 days after injection (randomised only).

Event		Low-dose		High-dose‡		P values*	
		$3x10^5$ pfu (n=38)	Placebo (n=5)	$\geq 10^7$ pfu (n=32)	Placebo (n=8)	$3x10^5$ pfu *vs* placebo	$3x10^5$ pfu *vs* $\geq 10^7$ pfu
Any adverse event	None (%)	4 (10·6)	1 (20)	0 (0·0)	1 (13)	0·372	0·189
	Mild (%)	11 (28·9)	3 (60)	7 (21·9)	5 (63)		
	Moderate (%)	17 (44·7)	1 (20)	16 (50·0)	2 (25)		
	Severe (%)	6 (15·8)	0 (0)	9 (28·1)	0 (0)		
Erythema**	None (%)	38 (100)	5 (100)	31 (96·9)	8 (100)	1	0·457
	Mild (%)	0 (0)	0 (0)	1 (3·1)	0 (0)		
	Moderate (%)	0 (0)	0 (0)	0 (0)	0 (0)		
Swelling / induration**	None (%)	37 (97·4)	5 (100)	30 (93·8)	8 (100)	1	0·589
	Mild (%)	1 (2·6)	0 (0)	2 (6·2)	0 (0)		
Pain**	None (%)	29 (76·3)	2 (40)	9 (28·1)	8·(100)	0·123	**0·0001**
	Mild (%)	9 (23·7)	3 (60)	22 (68·8)	0 (0)		
	Moderate (%)	0 (0)	0 (0)	1 (3·1)	0 (0)		
Objective fever	None (%)	37 (97·4)	5 (100)	22 (68·8)	8 (100)	1	**0·002**
	Mild (%)	1 (2·6)	0 (0)	10 (31·2)	0 (0)		
	Moderate (%)	0 (0)	0 (0)	0 (0)	0 (0)		
Subjective fever	None (%)	31 (81·6)	5 (100)	12 (37·5)	6 (75)	1	**0·001**
	Mild (%)	5 (13·2)	0 (0)	9 (28·1)	2 (25)		
	Moderate (%)	1 (2·6)	0 (0)	8 (25·0)	0 (0)		
	Severe (%)	1 (2·6)	0 (0)	3 (9·4)	0 (0)		
Chills	None (%)	24 (63·2)	3 (60)	13 (40·6)	8 (100)	0·836	0·120
	Mild (%)	8 (21·1)	1 (20)	6 (18·8)	0 (0)		
	Moderate (%)	4 (10·5)	1 (20)	10 (31·2)	0 (0)		
	Severe (%)	2 (5.3)	0 (0)	3 (9·4)	0 (0)		
Myalgia	None (%)	24 (63·2)	5 (100)	10 (31·2)	5 (63)	0·827	0·054
	Mild (%)	7 (18·4)	0 (0)	11 (34·4)	1 (13)		
	Moderate (%)	4 (10·5)	0 (0)	8 (25·0)	2 (25)		
	Severe (%)	3 (7·9)	0 (0)	3 (9·4)	0 (0)		
Headache	None (%)	26 (68·4)	4 (80)	13 (40·6)	5 (63)8	0·456	0·070
	Mild (%)	8 (21·1)	0 (0)	10 (31·2)	3 (38)		
	Moderate (%)	4 (10·5)	1 (20)	8 (25·0)	0 (0)		
	Severe (%)	0 (0)	0 (0)	1 (3.1)	0 (0)		
Fatigue	None (%)	14 (36·8)	3 (60)	12 (37·5)	6 (75)	0·545	0·640
	Mild (%)	11 (28·9)	2 (40)	6 (18·8)	2 (25)		
	Moderate (%)	11 (28·9)	0 (0)	13 (40·6)	0 (0)		
	Severe (%)	2 (5·3)	0 (0)	1 (3·1)	0 (0)		
Arthralgia§	None (%)	33 (86·8)	4 (80)	26 (81)	8 (100)	0·547	0·710
	Mild (%)	5 (13·2)	1 (20)	4 (13)	0 (0)		
	Moderate (%)	0 (0)	0 (0)	1 (3)	0 (0)		
	Severe (%)	0 (0)	0 (0)	1 (3)	0 (0)		

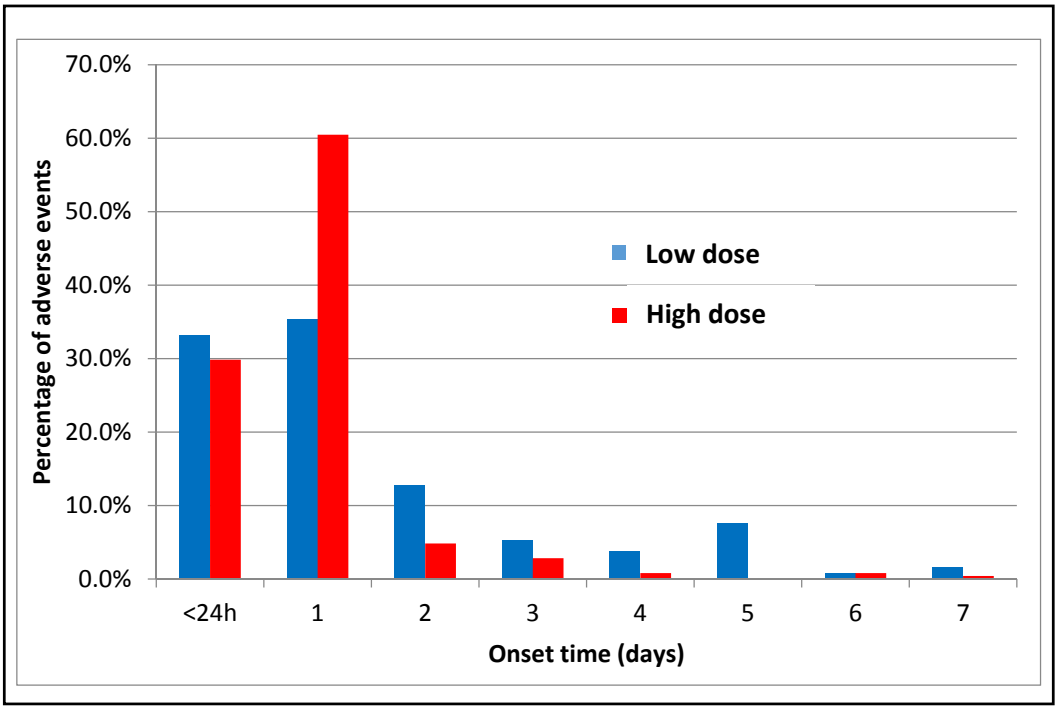
‡As previously described in reference 1.
*P values are for comparisons of solicited local and systemic adverse events between placebo recipients and vaccinees in low-dose and high-dose groups; Fisher's exact test was used.
**At injection site. §Arthralgia was solicited only in the low-dose group.

Table S5. Onset time of specific acute inflammatory symptoms occurring in the first seven days (reactogenicity).

Reactogenic event*	Day of onset						P values**
	Low dose (3x10^5 pfu)			High dose (≥ 10^7 pfu)‡			
	<24h	1 day	2-7 days	<24h	1 day	2-7 days	
Local pain	5 (45·5%)	3 (27·3%)	3 (27·3%)	27 (69·2%)	12 (30·8%)	0 (0·0%)	**0·012**
Subjective fever	3 (25·0%)	6 (50·0%)	3 (25·0%)	6 (18·8%)	24 (75·0%)	2 (6·3%)	0·156
Chills	4 (28·6%)	5 (35·7%)	5 (35·7%)	5 (18·5%)	20 (74·1%)	2 (7·4%)	**0·026**
Fatigue	14 (46·7%)	11 (36·7%)	5 (16·7%)	7 (22·6%)	21 (67·7%)	3 (9·7%)	**0·049**
Myalgia	4 (25%)	8 (50·0%)	4 (25·0%)	8 (23·5%)	24 (70·6%)	2 (5·9%)	0·169
Arthralgia	1 (16·7%)	2 (33·3%)	3 (50·0%)	1 (50·0%)	1 (50·0%)	0 (0·0%)	0·679
Headache	4 (28·6%)	4 (28·6%)	6 (42·9%)	9 (31·0%)	17 (58·6%)	3 (10·3%)	0·058
Nausea	1 (14·3%)	3 (42·9%)	3 (42·9%)	1 (7·7%)	11 (84·6%)	1 (7·7%)	0·104
Other	6 (33·3%)	4 (22·2%)	8 (44·4%)	8 (36·4%)	7 (31·8%)	7 (31·8%)	0·726

‡As previously described in reference 1. *Values are expressed as the absolute number of events occurring with onset during the given time period (%). **P values are for comparisons between low-dose and high-dose recipients of adverse event onset time in three categories (<24h, 1 day, 2-7 days); Fisher's exact test was used.

Figure S1. Onset time of all acute reactogenicity symptoms during the first week.



Blood counts per day and dose group (low-dose *vs* placebo recipients)

Table S6. Safety laboratory tests performed on screening days and follow-up visits.

Parameter	Days	All (n=64)	All placebo (n=13)	$3x10^5$ pfu (n=51)	P value*
Haemoglobin, g/L (SD)	0	142·8 (13·1)	142·1 (10·7)	143 (13·7)	0·881
	1	140·4 (13·1)	140·2 (11·3)	140·5 (13·7)	1
	3	140·2 (13·5)	140·2 (10·9)	140·2 (14·1)	0·815
	7	134·0 (13·2)	139·3 (10·6)	140·2 (13·8)	0·987
Platelets, G/L (SD)	0	246·6 (60·5)	234·4 (60·5)	249·7 (60·7)	0·605
	1	246·1 (64·1)	237·9 (66·0)	248·2 (64·2)	0·707
	3	238·0 (64·8)	241·9 (64·7)	237·0 (65·4)	0·588
	7	242·8 (67·5)	237·2 (71·0)	244·2 (67·2)	0·993
Lymphocytes, G/L (SD)	0	2·0 (0·6)	2 (0·7)	2·0 (0·6)	0·894
	1	1·7 (0·7)	2·0 (0·7)	1·6 (0·6)	**0·034**
	3	1·7 (0·6)	2·0 (0·8)	1·7 (0·6)	0·147
	7	2·1 (0·6)	2·1 (0·8)	2·1 (0·6)	0·822
Neutrophils, G/L (SD)	0	3·6 (1·6)	3·0 (1·0)	3·7 (1·7)	0·111
	1	4·1 (1·6)	3·0 (0·9)	4·4 (1·7)	**0·003**
	3	2·7 (1·0)	3·0 (0·8)	2·6 (1·1)	0·131
	7	3·1 (1·2)	2·9 (0·8)	3·2 (1·3)	0·940
Monocytes, G/L (SD)	0	0·5 (0·2)	0·5 (0·1)	0·5 (0·2)	0·488
	1	0·5 (0·2)	0·4 (0·1)	0·5 (0·2)	**0·026**
	3	0·6 (0·2)	0·5 (0·2)	0·7 (0·2)	**0·011**
	7	0·5 (0·2)	0·4 (0·1)	0·5 (0·2)	0·233

Values are expressed as means with standard deviation (SD) unless otherwise specified. *P values are for comparisons of haematological values, on each day, between participants receiving low-dose *vs* high-dose vaccine; the Mann-Whitney test was used.

Table S7. Differences between baseline and post-injection haematologic parameters.

Parameter	Days	All (n=64)	All placebo (n=13)	3x10^5 pfu (n=51)	P value*
Haemoglobin, g/L (SD)	1 *vs* 0	-2·38 (4·6)	-1·9 (4·2)	-2·5 (4·7)	0·380
	3 *vs* 0	-2·7 (5·1)	-1·9 (4·9)	-2·8 (5·2)	0·254
	7 *vs* 0	-2·8 (5·5)	-2·8 (6·5)	-2·8 (5·3)	1
Platelets, G/L (SD)	1 *vs* 0	-0·5 (20·9)	3·5 (12·7)	-1·5 (22·5)	0·226
	3 *vs* 0	-8·6 (23·8)	7·5 (12·4)	-12·7 (24·4)	**0·0002**
	7 *vs* 0	-3·8 (29·0)	2·9 (18·6)	-5·5 (31)	0·131
Leukocytes, G/L (SD)	1 *vs* 0	0·2 (1·4)	-0·04 (0·6)	0·2 (1·56)	0·483
	3 *vs* 0	-1·1 (1·39)	0·1 (0·9)	-1·4 (1·4)	**0·0005**
	7 *vs* 0	-0·4 (1·5)	-0·02 (1·0)	-0·5 (1·6)	0·301
Lymphocytes, G/L (SD)	1 *vs* 0	-0·3 (0·5)	0·04 (0·4)	-0·4 (0·6)	**0·005**
	3 *vs* 0	-0·2 (0·5)	0·03 (0·4)	-0·3 (0·5)	**0·026**
	7 *vs* 0	0·1 (0·5)	0·1 (0·6)	0·1 (0·5)	0·822
Neutrophils, G/L (SD)	1 *vs* 0	0·5 (1·4)	0 (0·5)	0·7 (1·5)	**0·012**
	3 *vs* 0	-0·9 (1·4)	-0·01 (0·6)	-1·1 (1·4)	**0·001**
	7 *vs* 0	-0·5 (1·4)	-0·1 (0·7)	-0·6 (1·5)	0·300
Monocytes, G/L (SD)	1 *vs* 0	0 (0·1)	-0·06 (0·1)	0·02 (0·1)	**0·019**
	3 *vs* 0	0·1 (0·2)	0·02 (0·2)	0·1 (0·2)	**0·014**
	7 *vs* 0	-0·01 (0·2)	-0·04 (0·1)	-0·01 (0·2)	0·187

Values are expressed by means with standard deviation (SD).

Table S8. Comparison of haematological values in low-dose and high-dose vaccinees.

Analyses	Days	All (n=102	Low dose ($3x10^5$) (n=51)	High-dose ($\geq 10^7$)‡ (n=51)	p value*
Platelets, g/L (SD)	0	246·1 (54·0)	249·7 (60·7)	242·4 (46·7)	0·710
	1	235·9 (57·1)	248·2 (64·2)	223·7 (46·5)	0·065
	3	227·2 (56·5)	237 (65·4)	217·4 (44·5)	0·222
	7	239·2 (58·1)	244·2 (67·2)	234 (47·0)	0·627
Leukocytes, G/L (SD)	0	6·3 (1·8)	6·4 (2·1)	6·1 (1·6)	0·551
	1	6·2 (1·9)	6·7 (2.0)	5·8 (1·8)	**0·038**
	3	4·8 (1·5)	5·1 (1·5)	4·6 (1·4)	0·063
	7	5·8 (1·7)	5·9 (1·8)	5·6 (1·6)	0·411
Lymphocytes, G/L (SD)	0	2·0 (0·5)	2·0 (0·6)	2 (0·5)	0·454
	1	1·2 (0·6)	1·6 (0·6)	0·9 (0·4)	**<0·0001**
	3	1·8 (0·6)	1·7 (0·6)	1·8 (0·5)	0·082
	7	2·0 (0·5)	2·1 (0·6)	2·0 (0·5)	0·521
Neutrophils, G/L (SD)	0	3·6 (1·5)	3·7 (1·7)	3·4 (1·3)	0·251
	1	4·3 (1·6)	4·4 (1·7)	4·3 (1·6)	0·839
	3	2·4 (1·1)	2·6 (1·1)	2·1 (1·1)	**0·002**
	7	3·1 (1·3)	3·2 (1·3)	3·0 (1·2)	0·415
Monocytes, G/L (SD)	0	0·5 (0·2)	0·5 (0·2)	0·5 (0·2)	0·703
	1	0·5 (0·2)	0·5 (0·2)	0·6 (0·2)	0·565
	3	0·6 (0·2)	0·7 (0·2)	0·6 (0·2)	**0·029**
	7	0·5 (0·2)	0·5 (0·2)	0·5 (0·2)	0·457

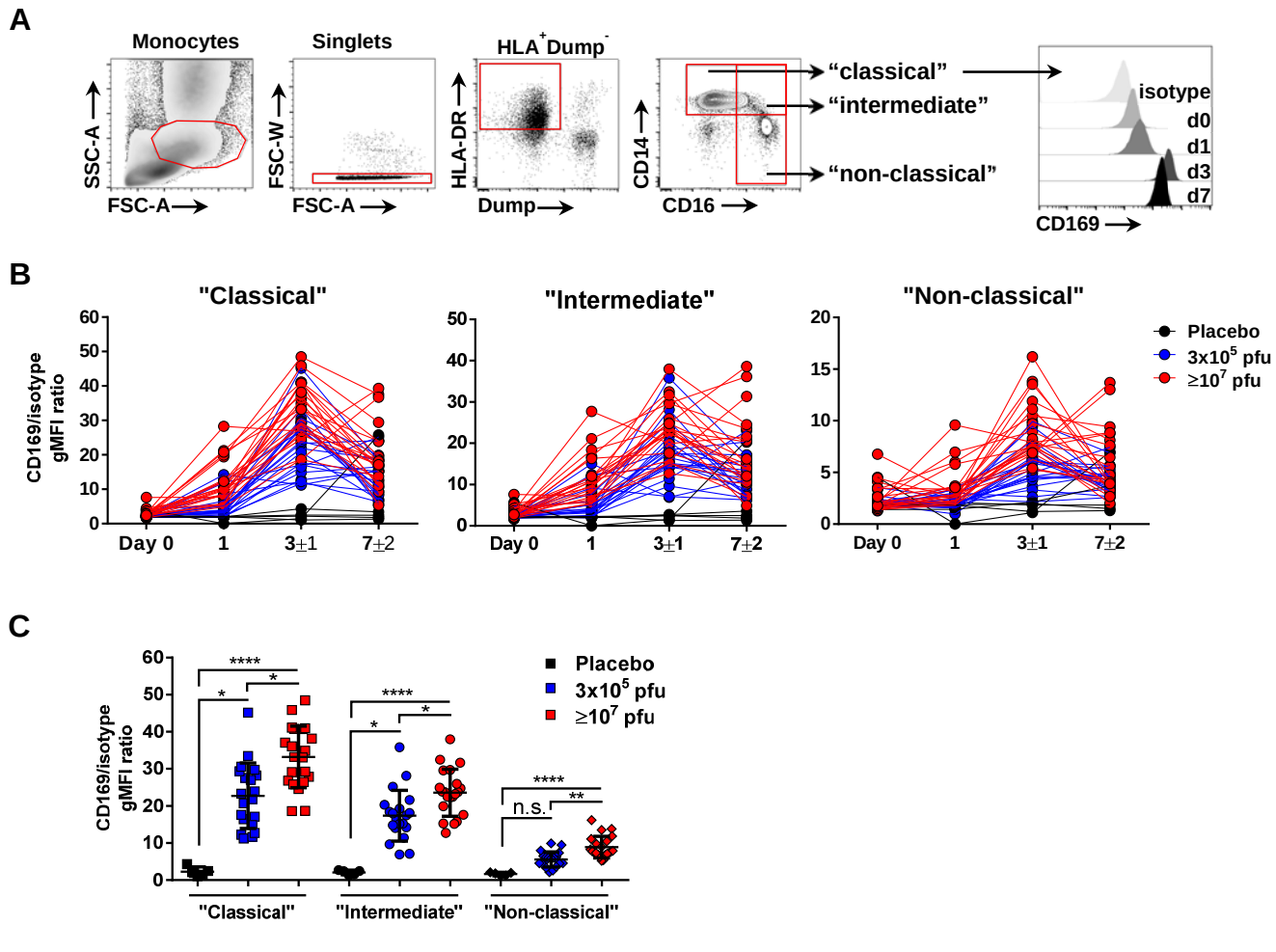
Values are expressed by means with standard deviation (SD) unless otherwise specified.

‡ As previously described in reference 1.

*P values are for comparisons of haematological values on each day between participants receiving low-dose *vs* high-dose vaccine; the Mann-Whitney test was used.

Monocyte activation by treatment group.

Figure S2. Upregulation of CD169 on monocyte populations following the receipt of rVSV-ZEBOV or placebo.



(A). The flow cytometric plots illustrate the gating strategy and the histogram shows a representative illustration of CD169/isotype control expression on classical monocytes.

(B). The CD169/isotype control gMFi ratio is shown for the classical, intermediate and non-classical blood monocyte populations[3] of 46 randomly selected volunteers, including 20 low-dose (blue dots), 21 high-dose (red dots) and 5 placebo recipients (black dots).

(C). The day-3 specific geometric mean fluorescence intensity (MFI) ratio of the CD169 expression in each treatment group was compared using the Kruskal-Wallis test. *p<0·05; **p<0·01; ****p<0·0001.

Viraemia

Table S9. Proportion of vaccinees with detectable rVSV RNA in plasma and copy numbers/ml per study group and day after injection.

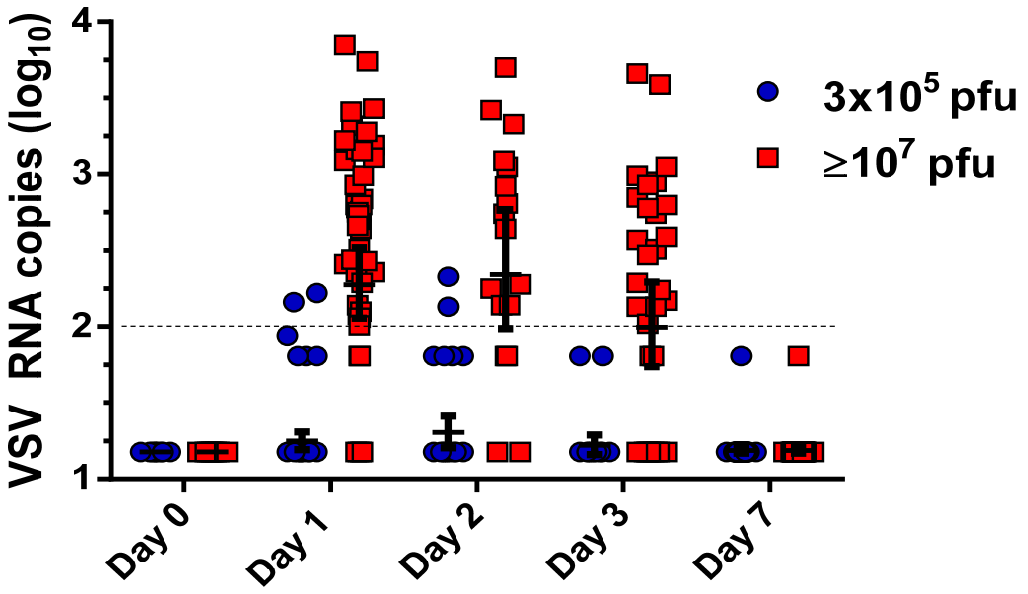
	All rVSV-ZEBOV vaccinees n=102		**rVSV-ZEBOV low-dose ($3x10^5$ pfu) n=51**		**rVSV-ZEBOV high-dose ($\geq 10^7$ pfu)* n=51**		**Comparison low-dose *vs* high-dose vaccinees** (p values)	
	Number positive (%)	**Copies/ml (median, (IQR))**	**Number positive (%)**	**Copies/ml (median, (IQR))**	**Number positive (%)**	**Copies/ml (median, (IQR))**	**Nb positive (Fisher test)**	**Copies/ml (Mann-Whitney test)**
Day 1	48/102 (47)	15 (15 – 309)	6/51 (12)	15 (15 – 15)	42/51 (82)	323 (65 – 912)	**<0·0001**	**<0·0001**
Day 3 (±1)	47/101 (47)	15 (15 – 191)	8/50 (16)	15 (15 – 15)	39/51 (76)	178 (65 – 676)	**<0·0001**	**<0·0001**
Day 7	2/95 (2)	15 (15 – 15)	1/46 (2)	15 (15 – 15)	1/51 (2)	15 (15 – 15)	1	0·953
Peak level (day 1-3)**	55/101 (54)	65 (15 – 501)	9/50 (18)	15 (15 – 15)	46/51 (90)	501 (138 – 1047)	**<0·0001**	**<0·0001**

Results are expressed as median number of copies/ml per group and time point. Values below the detection threshold (30 copies/ml) were arbitrarily given a value of 50% of this threshold (15 copies/ml) to allow for statistical analyses.

*In the absence of differences between values in recipients of 10^7 or $5x10^7$ pfu as previously described,[1] pooled results are presented.

** Peak value, whether measured at day 1, 2 or 3.

Figure S3. Illustration of rVSV RNA copy numbers in low- and high-dose vaccinees.



rVSV RNA copy numbers are expressed as $\log_{10}$ rVSV RNA copies/ml and are shown on days 0, 1, 3 and 7 for low-dose and high-dose vaccinees.

The dashed line indicates the limit of quantification. Positive values below this threshold were empirically attributed a 50% cut-off value.

Immunogenicity

Table S10. End-point geometric mean titres (GMT), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV measured by the USAMRIID ZEBOV-GP ELISA.

		N	GMT (95%CI)	Seropositivity (titre ≥ 50, n (%))	Seroresponse (≥ 4 fold, n (%))	P value, titre day 0 *vs* 28*	P value, seropositivity day 0 *vs* 28**
Placebo	Day 0	13	25 (-)	0 (0·0)	0 (0·0)	NA	NA
	Day 28	13	25 (-)	0 (0·0)			
$3x10^5$ pfu	Day 0	51	26 (24·5 to 27·6)	2 (3·9)	42 (82·4)	**<0·0001**	**<0·0001**
	Day 28	51	344·5 (229·7 to 516·4)	48 (94·1)			
$1x10^7$ pfu‡	Day 0	34	33·9 (26·6 to 43·4)	8 (23·5)	33 (97·1)	**<0·0001**	**<0·0001**
	Day 28	34	1064·2 (757·6 to 1495·1)	34 (100·0)			
$5x10^7$ pfu‡	Day 0	13	36·3 (26·1 to 50·5)	5 (38·5)	13 (100·0)	**0·002**	**0·008**
	Day 28	13	1780·1 (1048·3 to 3022·5)	13 (100·0)			
$1x10^7$ or $5x10^7$ pfu	Day 0	47	34·6 (28·4 to 42·1)	13 (27·7)	46 (97·9)	**<0·0001**	**<0·0001**
	Day 28	47	1227 (917·3 to 1641·2)	47 (100·0)			

Results are expressed as geometric means of endpoint titres (GMT) with 95% confidence intervals. Seropositivity is defined by an end-point titre ≥ 50. Values below this threshold were arbitrarily given a titre of 50% of this threshold to allow for statistical analyses. Seroresponse is defined by a ≥ 4-fold rise in endpoint titres.
‡ Previously described in reference 1.

* Titres at days 0 and 28 were compared using a Wilcoxon test for paired data.
**: Seropositivity rates at days 0 and 28 were compared using McNemar's test.
NA: not applicable

Table S11. Geometric mean concentrations (GMC), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV measured by a commercial ZEBOV-GP ELISA (ADI).

		N	GMT (95%CI)	Seropositivity (titre ≥ 100, n (%))	Seroresponse (≥ 4 fold, n (%))	P value, concentration day 0 *vs* 28*	P value, concentration day 0 *vs* 14*	P value, concentration day 14 *vs* 28*	P value, seropositivity day 0 *vs* 28**
Placebo	Day 0	13	66·0 (56·5 to 77·2)	0 (0·0)	0 (0·0)	**0·7190**	0.937	0.789	NA
	Day 14	13	61·5 (47·7 to 79·3)	0 (0·0)					
	Day 28	13	60·0 (46·4 to 77·5)	0 (0·0)					
3x10^5 pfu	Day 0	51	69·6 (59·7 to 81·0)	9 (17·6)	17 (33·3)	**<0·0001**	**<0.0001**	**<0.0001**	**<0·0001**
	Day 14	51	93·7 (79·0 to 111·2)	18 (35·3)					
	Day 28	51	241·4 (173·8 to 335·4)	37 (72·5)					
1x10^7 pfu‡	Day 0	35	51·2 (42·2 to 62·0)	5 (14·3)	19 (55·9)	**<0·0001**	**<0.0001**	**0.0025**	**<0·0001**
	Day 14	35	121·4 (92·6 to 159·1)	16 (45·7)					
	Day 28	34	342·3 (232·9 to 503·0)	31 (91·1)					
5x10^7 pfu‡	Day 0	16	63·1 (48·0 to 83·0)	1 (6·3)	8 (61·5)	**0·0002**	**0.0025**	**<0.0001**	**0·0015**
	Day 14	16	124·1 (85·8 to 179·6)	8 (50·0)					
	Day 28	13	392·8 (237·2 to 650·5)	12 (92·3)					
1x10^7 or 5x10^7 pfu	Day 0	51	54·6 (46·6 to 64·0)	6 (11·8)	27 (57·4)	**<0·0001**	**<0.0001**	**<0.0001**	**<0·0001**
	Day 14	51	122·2 (98·4 to 151·8)	24 (47·1)					
	Day 28	47	355·6 (261·0 to 484·4)	43 (91·5)					

Results are expressed as geometric mean concentrations (GMC) of ELISA units/ml with 95% confidence intervals. Seropositivity is defined by a concentration ≥ 100. Values below this threshold were arbitrarily given a titre of 50% of this threshold to allow for statistical analyses. Seroresponse is defined by a ≥ 4-fold rise in titres.

‡Previously described in reference 1.

*Concentrations were compared using Wilcoxon's test for paired data. **Seropositivity rates were compared using McNemar's test.

Table S12. Geometric mean concentrations (GMC), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV measured by whole-virion ELISA.

		N	GMT (95%CI)	Seropositivity (titre ≥ 50, n (%))	Seroresponse (≥ 4 fold, n (%))	P value, concentration day 0 *vs* 28*	P value, concentration day 0 *vs* 14*	P value, concentration day 14 *vs* 28*	P value, seropositivity day 0 *vs* 28**
	Day 0	13	538·6 (465·6 to 623·1)	1 (7·7)					
Placebo	Day 14	13	500 (-)	0 (0·0)	0 (0·0)	1	1.000	NA	0·317
	Day 28	13	500 (-)	0 (0·0)					
	Day 0	51	544·1 (495·3 to 597·8)	3 (5·9)					
$3x10^5$ pfu	Day 14	51	546·8 (494·6 to 604·6)	3 (5·9)	2 (3·9)	0·108	0.789	0.205	0·134
	Day 28	51	614·7 (524·3 to 720·5)	7 (13·7)					
	Day 0	34	557·2 (477·8 to 649·9)	2 (5·9)					
$1x10^7$ pfu‡	Day 14	34	615·1 (504·2 to 750·4)	4 (11·8)	6 (17·6)	**0·001**	0.584	**0.0001**	**0·002**
	Day 28	34	982·9 (721·1 to 1339·6)	14 (41·2)					
	Day 0	13	636·7 (459·2 to882·8)	2 (15·4)					
$5x10^7$ pfu‡	Day 14	13	707·2 (485·2 to 1030·7)	3 (23·1)	2 (15·4)	0·023	0.423	0.076	0·074
	Day 28	13	1285·1 (738·9 to 2235·3)	7 (53·8)					
	Day 0	47	578·2 (501·4 to 666·7)	4 (8·5)					
$1x10^7$ or $5x10^7$ pfu	Day 14	47	639·3 (536 to 762·5)	7 (14·9)	8 (17·0)	**<0·0001**	0.205	**<0.0001**	**0·0001**
	Day 28	47	1058·6 (807·9 to 1387·1)	21 (44·7)					

Results are expressed in arbitrary ELISA units (AEU)/ml with 95% confidence intervals. Seropositivity is defined by a concentration > 500 arbitrary ELISA units (AEU)/ml. Values below this threshold were arbitrarily given a titre of 50% of this threshold to allow for statistical analyses. Seroresponse is defined by a ≥ 4-fold rise in titres.

‡Previously described in reference 1. NA: not applicable.

* Concentrations at days 0 and 28 were compared using Wilcoxon's test for paired data. **Seropositivity rates at days 0 and 28 were compared using McNemar's test.

Table S13. Geometric mean concentrations (GMC), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV measured by a pseudovirion neutralization assay.

		N	GMT (95%CI)	Seropositivity (titre ≥ 20, n (%)	Seroresponse (≥ 4 fold, n (%))	P value concentration day 0 vs 28*	P value seropositivity day 0 vs 28**
Placebo	Day 0	13	10 (-)	0 (0·0)		NA	NA
	Day 28	13	10 (-)	0 (0·0)			
3x10^5 pfu	Day 0	51	10 (-)	0 (0.0)	30 (58·8)	**<0·0001**	**<0·0001**
	Day 28	51	35·4 (24·7 to 50·7)	30 (58·8)			
1x10^7 pfu‡	Day 0	34	10 (-)	0 (0.0)	24 (70·6)	**<0·0001**	**<0·0001**
	Day 28	34	99.1 (61.9 to 158.9)	30 (88.2)			
5x10^7 pfu‡	Day 0	14	10 (-)	0 (0·0)	13 (92·9)	**0·0001**	**0·0001**
	Day 28	14	231·8 (126·7 to 424·3)	14 (100·0)			
1x10^7 or 5x107 pfu‡	Day 0	48	10 (-)	0 (0·0)	37 (77·1)	**<0·0001**	**<0·0001**
	Day 28	48	127·0 (86·0 to 187·6)	44 (91.7)			

Results are expressed as geometric mean PsVNA50 neutralisation titres with 95% confidence intervals. Seropositivity is defined by a GMT > 20 of 2 replicates. Seroresponse is defined by a ≥ 4-fold rise in titres.

‡ Previously described in reference 1.

*Concentrations at days 0 and 28 were compared using Wilcoxon's test for paired data. **Seropositivity rates at days 0 and 28 were compared using McNemar's test.

Table S14. Comparison of day 28 results in recipients of $3x10^5$, 10^7 and $5x10^7$ pfu.

	GMT/GMC (Cuzick's test)	Seropositivity rates (Cochrane-Armitage test)	Seroresponse rates (Cochrane-Armitage test)
USAMRIID GP-ELISA	**<0·0001**	0·230	**0·016**
ADI GP-ELISA	**0·022**	**0·027**	**0·023**
Whole virion ELISA	**0·0005**	**0·0008**	0·098
PsVNA50	**<0·0001**	**0·0001**	**<0·0001**

Day 28 GMT/GMC, seroposivity rates and seroresponse rates were compared among recipients of $3x10^5$, 10^7 and $5x10^7$ pfu using Cuzick's and Cochrane-Armitage tests (as indicated) as appropriate to compare three independent groups. Results indicate that GMT/GMCs are significantly different, and whether seropositivity/seroresponse rates are significantly distinct or not.

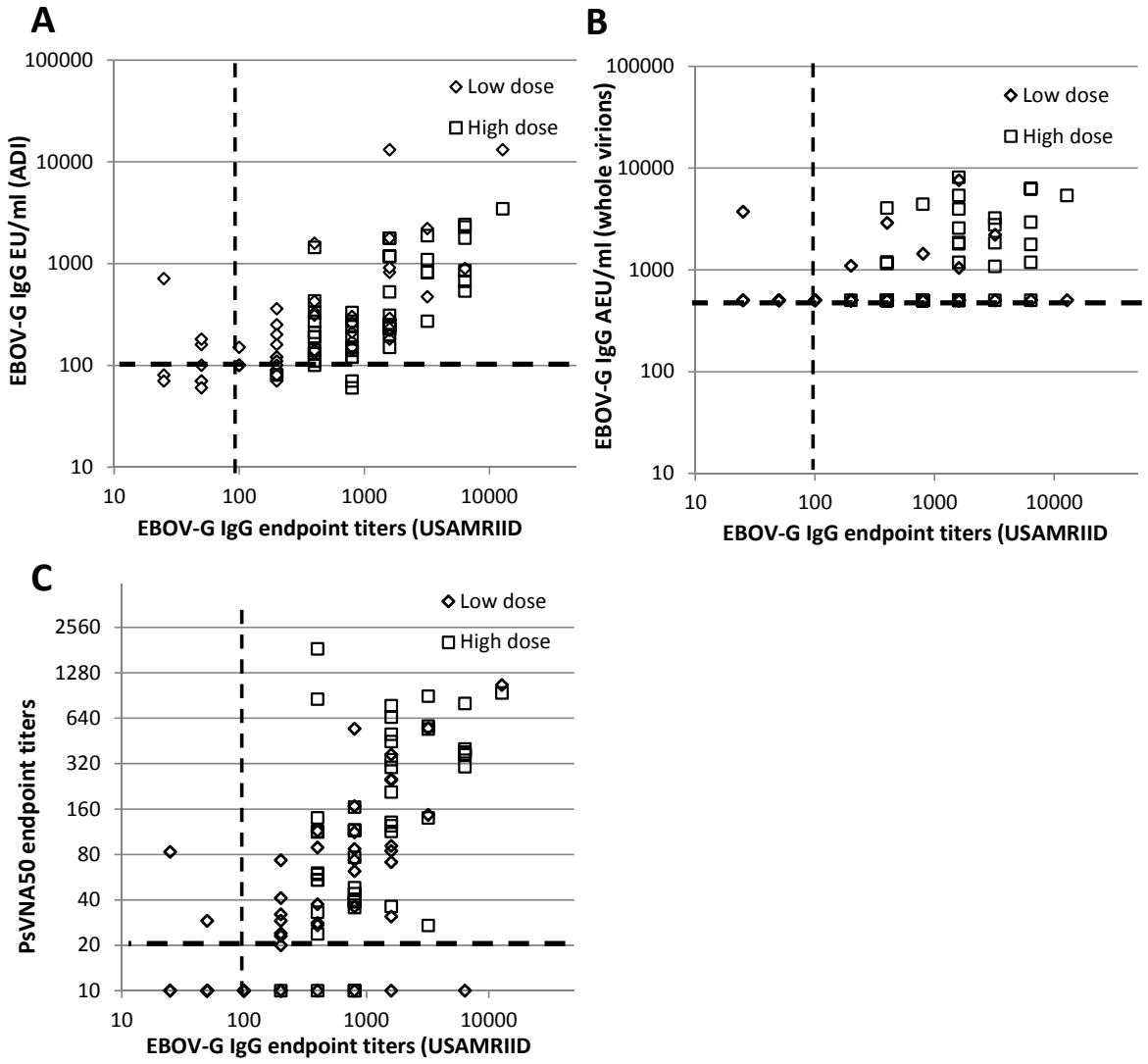
Table S15. Comparison of day 28 results between recipients of $3x10^5$ *vs* $\geq 10^7$ pfu.

	GMT/GMC (Mann-Whitney)	Seropositivity rates (Chi2)	Seroresponse rates (Fisher)
USAMRIID GP-ELISA	**<0·0001**	0·244	**0·017**
ADI GP-ELISA	**0·0006**	**0·031**	**0·025**
Whole-virion ELISA	**0·0320**	**0·002**	**0·045**
PsVNA50	**<0·0001**	**0·0004**	**0·0002**

Comparison of day 28 GMT/GMC, seroposivity rates and seroresponse rates were compared among recipients of $3x10^5$ and $\geq 10^7$ pfu (pooling recipients of 10^7 and $5x10^7$ pfu), using the Wilcoxon, Chi2 and Fisher tests as appropriate to compare two independent groups. This indicates that pooling the high-dose vaccinees into a single group does not affecting the statistical significance for the differences between low and high-dose vaccinees.

Figure S4. Correlation analyses between assays.

Correlation (correlation coefficients of Spearman) was assessed among day-28 results of all low-dose and high-dose vaccinees (placebo excluded).



In low-dose vaccinees, the correlation coefficients were:

- GP ELISA USAMRIID *vs* G ELISA ADI : ρ=0·71 (**p<0·0001**)
- GP ELISA USAMRIID *vs* whole-virion ELISA: ρ=0·15 (p=0·3071)
- GP ELISA USAMRIID *vs* PsVNA50: ρ=0·64 (**p<0·0001**)

In high-dose vaccinees, previously described in [1], the correlation coefficients were:

- GP ELISA USAMRIID *vs* G ELISA ADI : ρ=0.68 (**p<0·0001**)
- GP ELISA USAMRIID *vs* whole-virion ELISA : ρ=0·54 (**p<0·0001**)
- GP ELISA USAMRIID *vs* PsVNA50: ρ=0·55 (**p<0·0001**)

Table S16. Determinants of day-28 antibody titres (GMT/GMC) among low-dose vaccinees.

A significant correlation was observed only between monocytosis and the Day-28 GMC assessed with the least sensitive assay (whole-virion ELISA).

	GP ELISA – USAMRIID		**GP ELISA – ADI**	
	RGM (95%CI)	**P value**	**RGM (95%CI)**	**P value**
Age (per 10 years)	1·34 (0·94 to 1·90)	0·108	1·33 (1·01 to 1·76)	0·051
Gender (male)	1·40 (0·62 to 3·16)	0·421	1·41 (0·73 to 2·73)	0·310
Peak viraemia (per log_{10})	1·63 (0·46 to 5·76)	0·446	0·60 (0·22 to 1·66)	0·328
Lymphopaenia (per unit)	0·57 (0·12 to 2·72)	0·481	0·79 (0·22 to 2·85)	0·725
Monocytosis (per unit)	1·37 (0·54 to 3·47)	0·509	2·04 (0·98 to 4·24)	0·062
Lymphocytes: day-1 counts (per unit)	0·91 (0·47 to 1·74)	0·767	0·90 (0·53 to 1·52)	0·696
Monocytes: day-3 counts (per unit)	1·01 (0·11 to 9·58)	0·991	1·76 (0·29 to 10·81)	0·975
Number of AE (per AE)	1·00 (0·81 to 1·23)	0·993	1·00 (0·84 to 1·19)	0·976
	Whole-virion ELISA		**PsVNA50**	
	RGM (95%CI)	**P value**	**RGM (95%CI)**	**P value**
Age (per 10 years)	1·05 (0·91 to 1·21)	0·507	1·05 (0·77 to 1·45)	0·746
Gender (male)	0·79 (0·58 to 1·09)	0·155	1·19 (0·58 to 2·46)	0·637
Peak viraemia (per log_{10})	0·75 (0·46 to 1·22)	0·252	1·33 (0·44 to 4·04)	0·617
Lymphopaenia (per unit)	1·16 (0·63 to 2·16)	0·631	1·78 (0·79 to 4·03)	0·172
Monocytosis (per unit)	1·50 (1·06 to 2·12)	**0·028**	0·45 (0·11 to 1·79)	0·264
Lymphocytes: day-1 counts (per unit)	0·92 (0·71 to 1·19)	0·531	1·40 (0·62 to 3·18)	0·426
Monocytes: day-3 counts (per unit)	0·71 (0·29 to 1·70)	0·442	0·85 (0·48 to 1·51)	0·587
Number of AE (per AE)	0·97 (0·90 to 1·06)	0·526	0·97 (0·13 to 7·10)	0·976

Associations with day-28 antibody titres in low-dose vaccinees. Associations were assessed by unadjusted ratios of geometric means (RGM). For continuous variables (age, viraemia, haematological values, and number of AE), the RGM represents the ratio of the geometric means per unit (age: per 10 years; viraemia: per log_{10}). As an illustrative example, the geometric mean of EBOV-GP antibodies assessed by GP ELISA (USAMRIID) is multiplied by 1.34 for each 10 additional years of age. For categorical variables (gender), the RGM is ratio of the geometric mean between male and female.

Table S17. Determinants of day-28 antibody titres among all vaccinees, after adjustment for vaccine dose.

A significant correlation was observed only between lymphopaenia and the day-28 GMC assessed with the least sensitive assay (whole-virion ELISA) and PsVNA50.

	GP ELISA USAMRIID		**GP ELISA ADI**	
	RGM (95%CI)	**P value**	**RGM (95%CI)**	**P value**
Age (per 10 years)	1·19 (0·95 to 1·48)	0·131	1·19 (0·98 to 1·45)	0·085
Gender (male)	1·08 (0·64 to 1·80)	0·777	1·08 (0·68 to 1·72)	0·738
Peak viraemia (per log10)	1·08 (0·65 to 1·81)	0·762	1·18 (0·74 to 1·88)	0·482
Lymphopaenia (per unit)	1·15 (0·40 to 3·32)	0·799	1·40 (0·54 to 3·65)	0·489
Monocytosis (per unit)	1·40 (0·77 to 2·55)	0·279	1·53 (0·89 to 2·62)	0·126
Lymphocytes: day-1 counts (per unit)	1·19 (0·75 to 1·91)	0·460	1·13 (0·74 to 1·73)	0·577
Monocytes: day-3 counts (per unit)	2·43 (0·66 to 9·02)	0·187	2·27 (0·70 to 7·40)	0·177
Number of AE (per AE)	0·97 (0·86 to 1·09)	0·584	1·01 (0·90 to 1·13)	0·879
Grade 3 AE	0·76 (0·37 to 1·55)	0·447	1·01 (0·53 to 1·93)	0·983
	Whole-virion ELISA		**PsVNA50**	
	RGM (95%CI)	**P value**	**RGM (95%CI)**	**P value**
Age (per 10 years)	1·00 (0·87 to 1·14)	0·955	1·05 (0·84 to 1·32)	0·654
Gender (male)	0·93 (0·68 to 1·27)	0·633	1·15 (0·68 to 1·96)	0·596
Peak viremia (per log10)	0·94 (0·69 to 1·29)	0·699	1·44 (0·85 to 2·42)	0·175
Lymphopaenia (per unit)	2·07 (1·10 to 3·89)	**0·026**	2·36 (1·31 to 4·23)	**0·005**
Monocytosis (per unit)	1·17 (0·81 to 1·69)	0·407	1·13 (0·38 to 3·41)	0·825
Lymphocytes: day-1 counts (per unit)	1·21 (0·91 to 1·61)	0·184	1·89 (1·04 to 3·46)	0·041
Monocytes: day-3 counts (per unit)	1·68 (0·76 to 3·74)	0·207	1·10 (0·68 to 1·80)	0·691
Number of AE (per AE)	0·98 (0·90 to 1·05)	0·537	2·59 (0·68 to 9·79)	0·165
Grade 3 AE	0·71 (0·46 to 1·09)	0·122	0·94 (0·83 to 1·07)	0·346

Associations with day-28 antibody titres in all vaccinees. Associations were assessed by ratios of geometric mean (RGM) adjusted for the dose of vaccine. For continuous variables (age, viraemia, haematological values, and number of AE), the RGM represents the ratio of the geometric means per unit (age: per 10 years; viraemia: per $\log_{10}$). As an illustrative example, the geometric mean of EBOV-GP antibodies assessed by GP ELISA (USAMRIID) is multiplied by 1.19 for each 10 additional years of age. For categorical variables (gender), the RGM is ratio of the geometric mean between male and female. All RGM are derived from a multivariate linear regression model with dose (in 3 categories) as independent variable. Thus, the RGM shown in the tables are adjusted for the dose.

Determinants of risks for arthritis

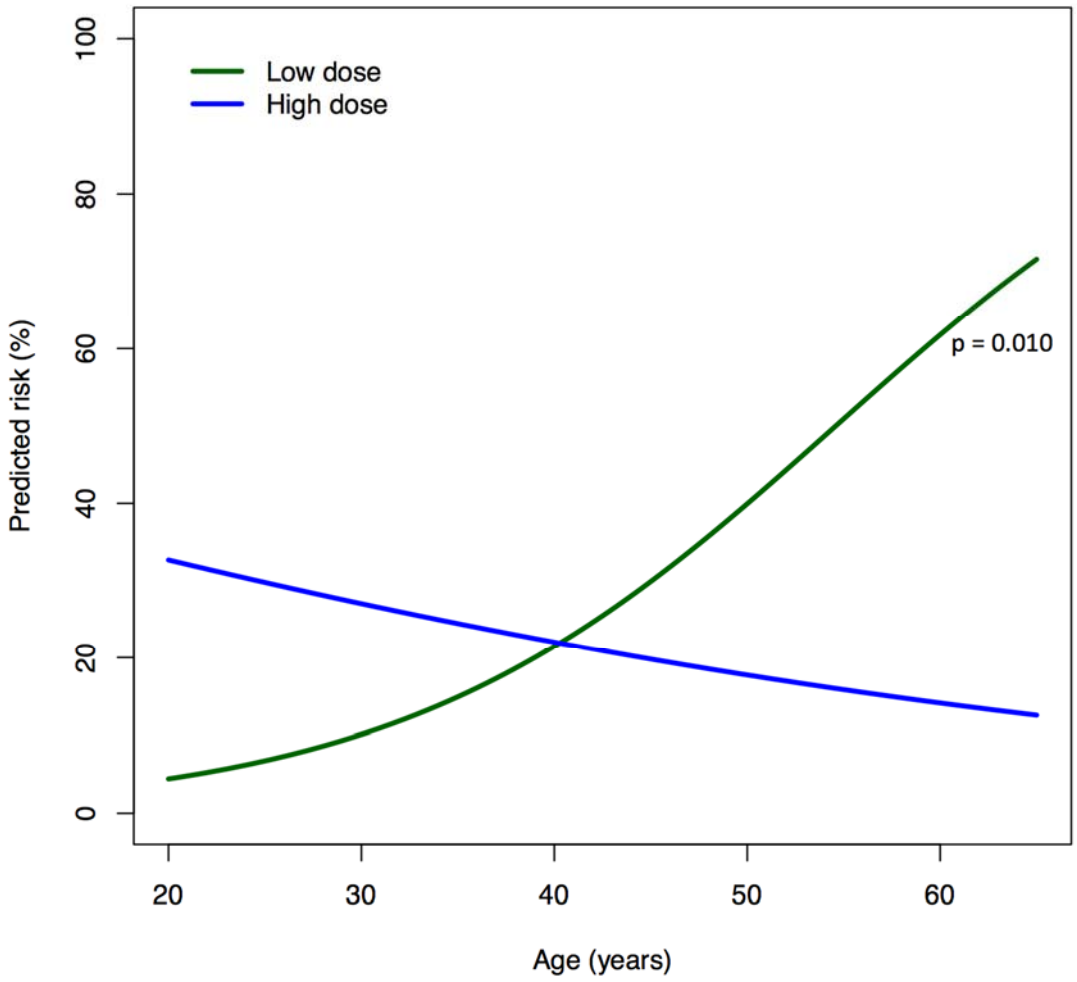
Table S18. Determinants of risks for arthritis in low-dose vaccinees.

Increasing age emerged as a significant risk factor for post-vaccination arthritis. Acute reactogenicity, including fever and/or arthralgia (which were rare in low-dose vaccinees), did not.

	Arthritis		
	No (n=38)	**Yes (n=13)**	**P value***
rVSV, log10 copy number/mL, median (IQR)	1·18 (1·18-1·18)	1·18 (1·18-1·18)	0·467
Age, years, median (IQR)	36·7 (29·7-42·7)	51·8 (47·3-54·0)	**0·006**
Lymphopaenia, median (IQR)	0·85 (0·64-0·99)	0·71 (0·57-0·86)	0·140
Monocytosis, median (IQR)	1·37 (1·19-1·49)	1·19 (1·07-1·38)	0·305
Gender			
Female, n (%)	20 (52·6)	7 (53·8)	1
Male, n (%)	18 (47·4)	6 (46·2)	
Viraemia (at days 1 or 3)			
rVSV<30 copies/ml	31 (83·8)	10 (76·9)	0·680
rVSV≥30 copies/ml	6 (16·2)	3 (23·1)	

*P values are for comparisons of pre-defined factors in low-dose vaccinees with and without arthritis, using the Mann-Whitney test for continuous variables and Chi-square or Fisher's exact test for categorical variables.

Figure S5. Predicted risks of arthritis with increasing age of vaccinees.



In a logistic regression model including all vaccinees (low- and high-dose), increasing age was significantly associated with the risk of arthritis in low-dose but not in high-dose vaccinees.

Reference list

1. Agnandji ST, Huttner A, Zinser ME et al. Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe - Preliminary Report. *N Engl J Med* 2015.
2. Kwissa M, Nakaya HI, Oluoch H et al. Distinct TLR adjuvants differentially stimulate systemic and local innate immune responses in nonhuman primates. *Blood* 2012; **119**: 2044-55.
3. Ziegler-Heitbrock L, Hofer TP. Toward a refined definition of monocyte subsets. *Front Immunol* 2013; **4**: 23.